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Expression of cytochromes P450 1A1 and 1B1 in human lung from smokers, non-smokers, and ex-smokers

James H. Kim,[a] Mark E. Sherman,[b] Frank C. Curriero,[c] F. Peter Guengerich,[d] Paul T. Strickland,[a] and Thomas R. Sutter[a,e,*]

[a] *Department of Environmental Health Sciences, The Johns Hopkins University School of Hygiene and Public Health, Baltimore, MD 21205, USA*
[b] *Department of Pathology, The Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA*
[c] *Department of Biostatistics, The Johns Hopkins University School of Hygiene and Public Health, Baltimore, MD 21205, USA*
[d] *Department of Biochemistry and Center in Molecular Toxicology, Vanderbilt University School of Medicine, Nashville, TN 37232, USA*
[e] *W. Harry Feinstone Center for Genomic Research, University of Memphis, Memphis, TN 38152, USA*

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Abstract

Cytochromes P450 1A1 and 1B1 are known to bioactivate procarcinogens such as polycyclic aromatic hydrocarbons (PAHs) found in cigarette smoke and are inducible via an Ah receptor-mediated mechanism. The aim of this study was to examine the levels of expression of CYP1A1 and CYP1B1 in samples of lung from smokers (n = 18), non-smokers (n = 7), and ex-smokers (n = 7). Using immunoglobulin preparations of highly specific polyclonal antibodies and immunoblot analysis of microsomes from lung tissues, we determined the specific content for CYP1A1 and CYP1B1. For CYP1A1, we found median expression levels of 15.5 pmol/mg microsomal protein in smokers, 6.0 pmol/mg microsomal protein in non-smokers, and 19.0 pmol/mg microsomal protein in ex-smokers. The difference in median expression levels of smokers and ex-smokers compared to non-smokers was statistically significant. For CYP1B1, we found median expression levels of 1.8 pmol/mg microsomal protein in smokers, 1.0 pmol/mg microsomal protein in non-smokers, and 4.4 pmol/mg microsomal protein in ex-smokers. The difference in median expression levels between ex-smokers and non-smokers was statistically significant. These results suggest that levels of expression of CYP1A1 and CYP1B1 protein in lung tissues from smokers and ex-smokers are quantitatively greater than in non-smokers. By immunohistochemical analysis, we demonstrated the expression of CYP1A1 and CYP1B1 in normal human alveolar type I and II cells, ciliated columnar epithelial cells lining bronchoalveolar airways, and alveolar macrophages. These results confirm that CYP1A1 is expressed in normal human lung, appears to be induced in smokers, and show interindividual variation; the similar characteristics of CYP1B1 are demonstrated.

Keywords: Cytochrome P4501B1; Lung; Smoking; Polycyclic aromatic hydrocarbons

Introduction

Many carcinogens require bioactivation by endogenous enzymes to achieve their mutagenic and carcinogenic forms. Numerous carcinogens, such as polycyclic aromatic hydrocarbons (PAHs) and nitrosamines, are metabolized by cytochrome P-450 (CYP) to both mutagenic and non-mutagenic metabolites. Studies with benzo[*a*]pyrene (B[*a*]P) have shown that CYP enzymes are critical to the bioactivation of this procarcinogen to the postulated ultimate carcinogens, the diol-epoxides (Conney et al., 1994; Gelboin, 1980). Additionally, B[*a*]P is capable of inducing the expression of CYP through an Ah receptor-dependent mechanism, thereby increasing the rate of its own metabolism (Hankinson, 1995; Nebert and Gelboin, 1968; Whitlock, 1989).

CYP1 family enzymes are inducible by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and other PAHs through high affinity binding of the Ah receptor (Rendic and Di Carlo, 1997; Whitlock, 1999). The CYP1A subfamily consists of two members, of which CYP1A1 catalyzes the metabolism of B[*a*]P and many other PAHs, and CYP1A2 that shares some substrate overlap (Rendic and Di Carlo, 1997).

* Corresponding author. W. Harry Feinstone Center for Genomic Research, University of Memphis, 201 Life Science Building, 3774 Walker Avenue, Memphis, TN 38152. Fax: +1-901-678-4457.
E-mail address: tsutter@memphis.edu (T.R. Sutter).

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CYP1B1 is the only member of the 1B subfamily and has been shown to metabolize a large number of tobacco, environmental and dietary carcinogens, including B[*a*]P (Hayes et al., 1996; Kim et al., 1998; Shimada et al., 1996a, 1996b; Sutter et al., 1994). Dibenzo[*a,l*]pyrene (DB[*a,l*]P) and 7,12-dimethylbenz[*a*]anthracene (DMBA) are sterically hindered PAHs bioactivated preferentially by CYP1B1 to ultimate carcinogenic forms (Buters et al., 1999; Luch et al., 1998; Otto et al., 1992; Shimada et al., 1996a). Both of these enzymes, CYP1A1 and CYP1B1, may play important roles in the bioactivation of tobacco smoke carcinogens.

Extra-hepatic expression of cytochromes P450 in humans is of importance in determining the metabolic activation of procarcinogens and xenobiotics. Unlike CYP1A2, which is primarily a hepatic enzyme, CYP1A1 has been found in many extra-hepatic tissues at low basal levels (Rendic and Di Carlo, 1997). In addition, CYP1A1 is highly inducible, leading to speculation that CYP1A1 may be the primary enzyme responsible for procarcinogen activation in extra-hepatic tissues (Okey, 1990). However, mRNA analysis has found CYP1B1 to be more widely distributed among human extra-hepatic tissues compared to CYP1A1 (Shimada et al., 1996a). In addition, CYP1B1 is also inducible through a similar Ah receptor-mediated mechanism as CYP1A1 (Spink et al., 1997; Sutter et al., 1991, 1994; Tang et al., 1996). Taken together, these data suggest that CYP1B1 may also play a major role in extra-hepatic procarcinogen metabolism along with CYP1A1.

Interest in human lung expression of CYP stems from the cause–effect relationship between tobacco smoking and lung cancer (Samet, 1994). It has been hypothesized that genetic variances in CYP expression, inducibility, or activity are responsible for individual susceptibility to cancer (Alexandrie et al., 1994; Nebert, 1991). CYP1A1 expression and activity has been extensively characterized in human tissues. However, most studies have focused on mRNA expression (Czerwinski et al., 1994; Willey et al., 1997). Because of minimal availability of reagents and high quality normal tissue samples, only a few studies have reported on positive CYP1A1 protein expression in human lung by immunoblot and immunohistochemical techniques (Anttila et al., 1991, 1992, 1997; Kivisto et al., 1995; Saarikoski et al., 1998).

CYP1B1 in humans has not been as well studied. In one immunochemical study, CYP1B1 protein was detected in human tumors but not in corresponding normal tissues (Murray et al., 1997). This conflicts with current knowledge about CYP1B1 mRNA expression, which has been found extensively in extra-hepatic human tissues (Shimada et al., 1996a; Sutter et al., 1994). In this report, we have obtained normal human lung tissues from donors representing smokers, non-smokers, and ex-smokers to characterize the protein expression and cellular localization of CYP1B1 and CYP1A1. The results described in this manuscript are a direct extension of the three decades of pioneering research conducted by Dr. Bresnick in the area of cytochrome P450 regulation in response to PAHs. His work contributed greatly to our current understanding of the mechanisms by which PAHs affect aryl hydrocarbon hydroxylase activity and the CYP1 enzymes that catalyze this reaction.

Table 1
Summary of individual data for CYP1B1 and CYP1A1 expression in human lung tissue samples

Designation by smoking status[a]	Age/gender/smoking history/cancer[b]	CYP1B1 (pmol/mg microsomal protein)	CYP1A1 (pmol/mg microsomal protein)
S-1	67/M/52 pky/adenocarcinoma	12.7	289.7
S-2	?/?/8 pky/lung cancer	6.3	137.0
S-3	62/F/>20 pky/adenocarcinoma	1.1	38.7
S-4	45/F/smoker/lung cancer	2.2	18.2
S-5	52/M/40 pky/adenocarcinoma	3.9	23.7
S-6	69/M/>40 pky/sq. cell carcinoma	2.1	13.9
S-7	75/M/smoker/sq. cell carcinoma	7.7	46.5
S-8	62/M/>20 pky/adenocarcinoma	5.8	16.2
S-9	44/M/smoker/sq. cell carcinoma	4.5	18.1
S-10	17/M/2 pky/autopsy, gunshot wound	1.3	14.8
S-11	30/F/>5 pky/autopsy, hanging	2.1	21.3
S-12	54/F/48 pky/non-small cell carcinoma	1.4	6.0
S-13	?/?/smoker/cancer	1.4	9.0
S-14	53/M/smoker/adenocarcinoma	0.9	13.9
S-15	68/F/smoker/adenocarcinoma	0.5	9.8
S-16	67/F/30 pky/sq. cell carcinoma	0.9	9.7
S-17	63/M/smoker/adenocarcinoma	1.6	8.7
S-18	73/F/smoker/non-small cell carcinoma	0.4	5.5
NS-1	82/F/non-smoker/metastasis to lung	2.3	5.2
NS-2	?/?/non-smoker/?	2.3	10.3
NS-3	60/F/non-smoker/autopsy, hemorrhage	0.9	8.9
NS-4	24/M/non-smoker/autopsy, motor accident	0.3	5.5
NS-5	16/F/non-smoker/autopsy, hemorrhage	0.9	6.6
NS-6	55/M/non-smoker/autopsy, cerebral vasospasm	1.0	4.3
NS-7	17/F/non-smoker/autopsy, head trauma	1.3	10.7
X-1	70/M/160 pky; quit 10y/ non-small cell carc.	4.9	16.1
X-2	73/F/76 pky; quit 19y/cancer	4.4	85.1
X-3	62/F/60 pky; quit 10y/lymphoma	3.6	15.1
X-4	70/M/72 pky; quit 9y/ adenocarcinoma	5.9	35.5
X-5	74/M/25 pky; quit 27 y/ non-small cell carc.	6.2	33.2
X-6	76/M/15 pky, quit 1y/carc.	1.7	8.4
X-7	59/F/20 pky; quit 6y/lung chondroma	2.9	19.0

[a] S, smoker; NS, non-smoker; X, ex-smoker. These designations are based on information given to hospital or organ donor agency as part of the family history.
[b] ?, unknown; pky, pack years.

Methods

Isolation of human lung microsomes. Frozen tissue samples were obtained either as anatomical gifts from the International Institute for the advancement of Medicine (Scranton, PA) or as discarded normal tissue adjacent to tissue surgically removed at the Vanderbilt University Medical Center. All samples were collected under protocols approved by the Committee on Human Research at the respective institutions. Sample information is provided in Table 1. In the case of surgical samples, smoking status was self-reported. In the case of tissue donation, smoking status was reported by the donor's next of kin. The definition of exposure is defined as pack year, corresponding to 7300 cigarettes per year. The frozen tissue samples were processed using a Bessman tissue pulverizer (Fisher Scientific, Pittsburgh, PA) cooled in liquid nitrogen and placed into homogenization buffer (20 mM Tris acetate, pH 7.4, 0.14 M KCl, 1 mM DTT, 0.2 mM PMSF, 1 mM EDTA) at 10 ml/g of tissue. Subsequent microsomal preparation was performed in a cold room at 4 °C. Samples were homogenized in a Potter-Elvehjem homogenizer using 4–5 passes of the pestle attached to a Bellco homogenizer drive unit on a medium setting. The homogenates were fractionated by centrifugation for 20 min at 9000 × *g*, and the supernatant was further fractionated by centrifugation at 105 000 × *g* for 60 min to obtain a microsomal pellet and cytosolic supernatant. The supernatant was again fractionated by centrifugation at 183 000 × *g* to obtain an additional microsomal pellet. The microsomal pellets were resuspended in freezing buffer (10 mM Tris acetate, pH 7.4, 1 mM EDTA, 20% glycerol), combined, and briefly homogenized before storage at −85 °C for later analysis.

Antibodies. Development of rabbit anti-CYP1A1 and anti-CYP1B1 polyclonal antibodies was described previously (Walker et al., 1998, 1999). These antibodies were used in the following immunoblotting and immunohistochemical methods.

Immunoblotting. Microsomal samples were solubilized in sodium dodecyl sulfate sample dilution buffer and separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis on a 7.5% gel. Proteins were electrophoretically transferred to nitrocellulose membranes (Hybond ECL, Amersham, Piscataway, NJ) and probed for 1 h with rabbit anti-P4501B1 or anti-P4501A1 IgG at antibody concentrations of 5 μg/ml. Proteins were detected by incubation for 1 h with a horseradish peroxidase-linked secondary antibody (goat anti-rabbit IgG, 1:30 000, Promega, Madison, WI). Bound secondary antibody was detected by an enhanced chemiluminescent method (SuperSignal, Pierce, Rockford, IL). Cytochromes P450 1A1 and 1B1 were quantified using densitometric analysis and ImageGauge software (Fujifilm, Edison, NJ), and comparison with standard curves generated by using human recombinant CYP1A1 and CYP1B1 in human lymphoblastoid cell microsomes whose concentration was spectrophotometrically determined (CYP1A1 standard curve: 0.05, 0.1, 0.2, 0.3, 0.5 pmol, $r = 0.97$; CYP1B1: 0.05, 0.1, 0.2, 0.4 pmol, $r = 0.99$).

Immunohistochemistry. Sections of frozen human lung tissue were fixed in 10% formalin, paraffin-embedded, and were serially sectioned at 4-μm increments for immunohistochemical analysis. Anti-P4501B1 IgG was used at 0.3 μg/ml and anti-P4501A1 IgG was used at 1.0 μg/ml for anti-P4501A1 IgG. Slides were deparaffinized in xylene and rehydrated in ethanol and PBS. Endogenous peroxide activity was blocked using 3% hydrogen peroxide. Slides were then subjected to antigen retrieval by steaming for 12 min in citrate buffer pH 6.0. Normal goat serum was then applied to the slides as a blocking agent. Slides were incubated with primary antibody at 4 °C in a humidified chamber overnight.

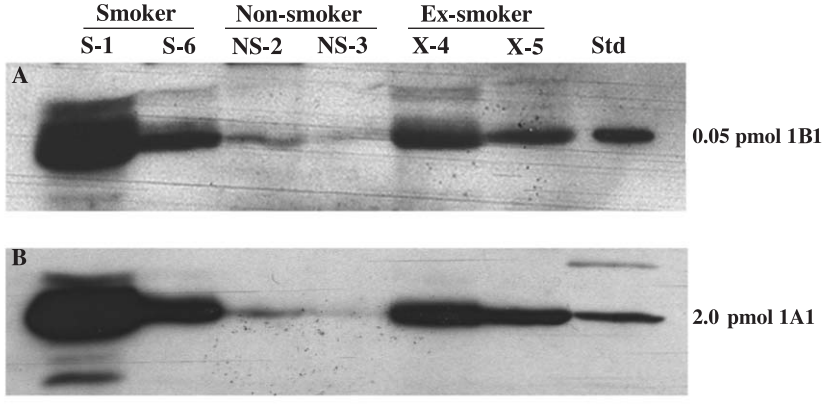

Fig. 1. Representative immunoblot showing CYP1B1 and CYP1A1 expression in human lung microsomes. (A) CYP1B1 immunoblot of microsomes from two smokers (S-1 and S-6), two non-smokers (NS-2 and NS-3), and two ex-smokers (X-4 and X-5). Recombinant CYP1B1, 0.05 pmol, was loaded as a standard in the far right lane. (B) CYP1A1 immunoblot of microsomes from two smokers (S-1 and S-6), two non-smokers (NS-2 and NS-3), and two ex-smokers (X-4 and X-5). Recombinant CYP1A1, 2.0 pmol, was loaded in the far right lane.

Table 2
Sample size, median, 25th and 75th percentiles of CYP1A1, and CYP1B1 for the smoker, non-smoker, and ex-smoker sample groups

	CYP1A1 (pmol/mg microsomal protein)			CYP1B1 (pmol/mg microsomal protein)		
	Smoker	Non-smoker	Ex-smoker	Smoker	Non-smoker	Ex-smoker
n	18	7	7	18	7	7
25th Percentile	9.7	5.3	15.3	1.1	0.9	3.1
Median	15.5	6.6	19.0	1.8	1.0	4.4
75th Percentile	23.7	9.9	34.9	4.5	2.0	5.6

Secondary antibody was supplied with the Vector Elite ABC kit (Vector, Burlingame, CA) and was used according to the recommendations of the manufacturer. Incubation with biotinylated goat anti-rabbit secondary IgG was performed for 1 h at room temperature, followed by incubation with a tertiary avidin–biotin complex for 30 min. Diaminobenzidine chromagen was used for detection of bound antibodies, and nuclei were counterstained with hematoxylin. Slides were dehydrated through increasing ethanol concentrations, cleared in xylene, and mounted.

Statistical methods. Nonparametric statistical methods (Conover, 1980; Hettmansperger, 1984) were applied to the CYP1A1 and CYP1B1 data to minimize the influence of potential outlier observations with the small sample sizes. Analysis of variance (ANOVA) using the Kruskal–Wallis rank sum test was used to determine if the median levels of CYP1A1 and CYP1B1 differ significantly across the smoker, non-smoker, and ex-smoker sample groups. Pairwise multiple comparisons based on Dunn's methods were also computed for significant ANOVA results (P value < 0.05)

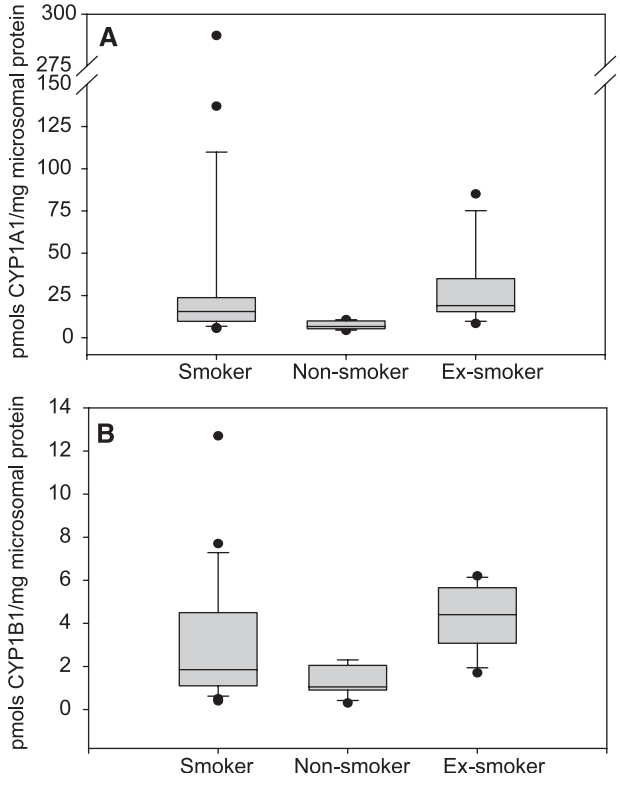

Fig. 2. Quantitative analysis of CYP in human lung microsomes from smokers, non-smokers, and ex-smokers. Box plot distributional summaries for (A) CYP1A1 and (B) CYP1B1 indicating the medians, the 25th and 75th percentiles by the boxes, the 10th and 90th percentiles by the whiskers, and outliers as individual points.

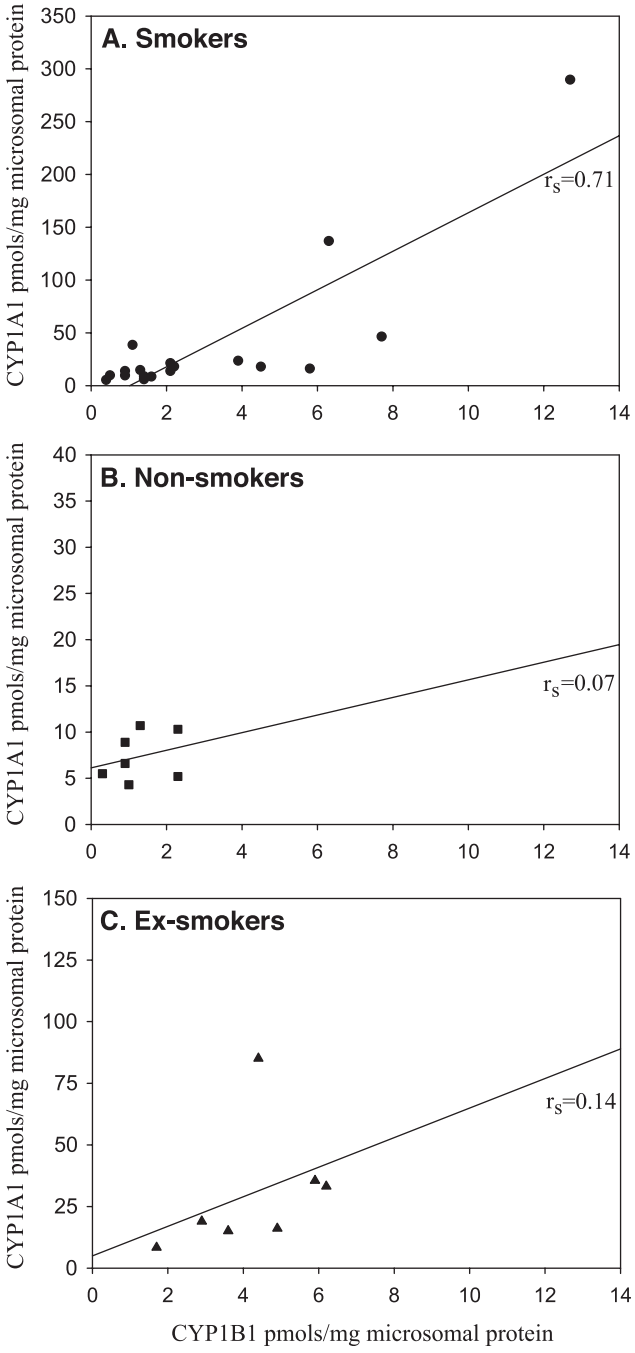

Fig. 3. Spearman's rank correlation, r_s, of CYP1B1 (x axis) and CYP1A1 (y axis) expression in human lung microsomes from (A) smoker, (B) non-smoker, and (C) ex-smoker. A least squares regression line is also included.

to determine which pairwise set of samples were significantly different. Sample sizes necessary to achieve statistically significant ANOVA and pairwise comparisons results for possible future studies were determined based on the methods presented in Cohen (1988).

The relationship between CYP1A1 and CYP1B1 was described using the Spearman's rank correlation (Hettmansperger, 1984), which is a nonparametric measure of association, and tested for statistical significance. All statistical computations were performed using SigmaStat (Jandel Corp., Chicago, IL).

Results

Expression of CYP1A1 and CYP1B1 was analyzed quantitatively by immunoblot. A representative immunoblot showing high and low CYP1A1 and CYP1B1 is presented in Fig. 1 from each of the three groups: smokers, non-smokers, and ex-smokers. CYP was detected in all microsomal samples analyzed. A summary of the individual data and smoking history is presented in Table 1. Levels of CYP1B1 protein (presented as picomole CYP1B1 protein per milligram microsomal protein) ranged from 0.4 to 12.7 in smokers; from 0.3 to 2.3 in non-smokers; from 1.7 to 6.2 in ex-smokers. Levels of CYP1A1 protein ranged from 5.5 to 289.7 in smokers; from 4.3 to 10.7 in non-smokers; from 8.4 to 85.1 in ex-smokers. Presented in Table 2 are the medians, 25th and 75th percentiles of the CYP1A1 and CYP1B1 data for the smoker, non-smoker, and ex-smoker sample groups. This information with more detail is shown graphically in Fig. 2 using box plot distributional summaries.

Results from the Kruskal–Wallis one-way ANOVA that indicate the median expression levels in both CYP1A1 and CYP1B1 are significantly different across the smoker, non-smoker, and ex-smoker groups; P values were 0.007 and 0.023 for CYP1A1 and CYP1B1, respectively. Follow-up pairwise comparisons showed that for CYP1A1, the median expression levels in smokers and ex-smokers were signifi-

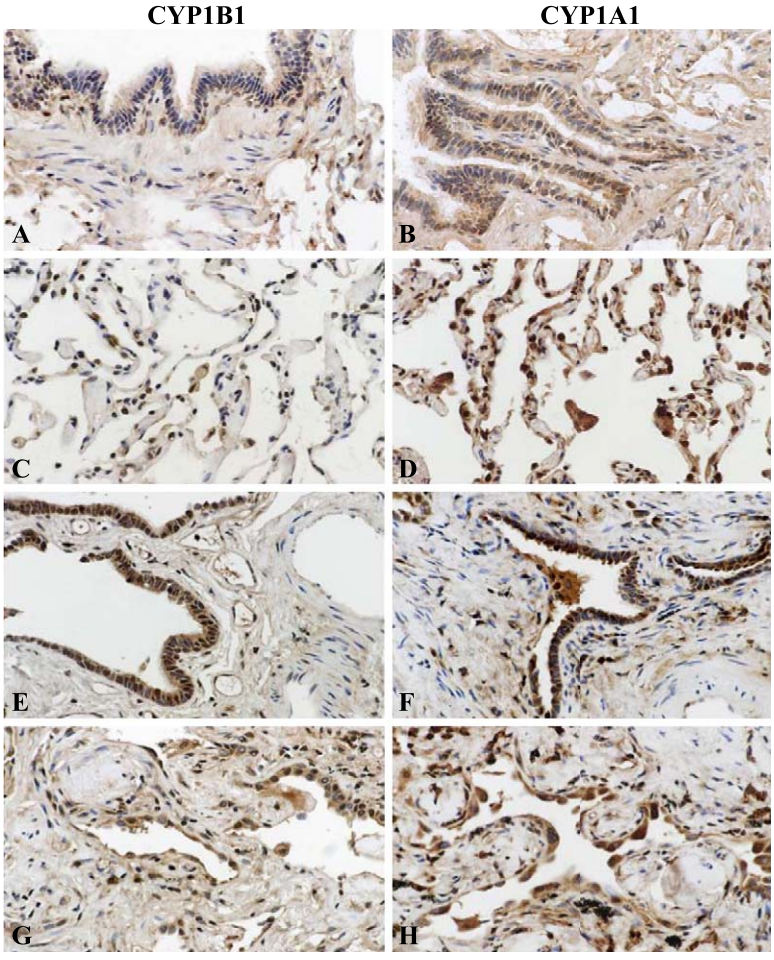

Fig. 4. Representative immunohistochemistry slides showing CYP1B1 and CYP1A1 expression in human lung. (A) CYP1B1 in non-smoker NS-3, (B) CYP1A1 in non-smoker NS-3, (C) CYP1B1 in non-smoker NS-2, (D) CYP1A1 in non-smoker NS-2, (E) CYP1B1 in smoker S-6, (F) CYP1A1 in smoker S-6, (G) CYP1B1 in smoker S-6, (H) CYP1A1 in smoker S-6.

cantly greater than the median level observed in non-smokers (P value < 0.05). The median expression level between smokers and ex-smokers for CYP1A1 was not significantly different. For CYP1B1, the median expression level for ex-smokers was significantly greater than the median level for non-smokers (P value < 0.05); however, comparisons of smokers with ex-smokers and non-smokers were not significantly different. As previously reported, the CYP1A1 antibody used in this study cross-reacts with CYP1A2, which shows an electrophoretic mobility that is distinct from CYP1A1 (Walker et al., 1998). However, no CYP1A2-associated immunoreactivity was detected for exposures in the linear range of our standard curves used for quantitation. Beyond this range, multiple cross-reacting and potentially nonspecific bands could be observed (Fig. 1).

Fig. 3 shows plots of CYP1A1 versus CYP1B1 levels for the smoker, non-smoker, and ex-smoker sample groups along with the calculated Spearman's rank correlation coefficient r_s. For smokers, these results indicate a positive association between levels of CYP1A1 and CYP1B1 (r_s = 0.71, P value = 0.001). No significant association was found between CYP1A1 and CYP1B1 levels for the non-smoker and ex-smoker groups.

CYP1A1 and CYP1B1 expression in human lung was also analyzed by immunohistochemical procedures (Fig. 4). Panels A, C, E, and G are lung slides stained with anti-CYP1B1 antibody. Panels B, D, F, and H are lung slides stained with anti-CYP1A1 antibody. Panels A, B, C, and D are lung slides obtained from a non-smoker (A, B, NS-3; C, D, NS-2); panels E, F, G, and H are from a smoker (S-6). CYP expression was detected in every sample analyzed. However, staining for CYP1B1 and CYP1A1 in non-smokers is much less intense compared to the staining in smokers. CYP1A1 and CYP1B1 protein was observed in alveolar type I and type II cells (Panels C, D, G, and H), ciliated columnar epithelial cells (Panels A, B, E, and F), alveolar macrophages, and possible Clara cells, which are difficult to clearly identify. The ciliated columnar epithelial cells appear to possess the most intense staining for both CYP1A1 and CYP1B1. Slides stained with pre-immune serum IgG were negative (data not shown).

Discussion

Measurements of the amount of CYP1A1 and CYP1B1 protein in human lung were performed on isolated microsomes and presented as picomole CYP per milligram microsomal protein. Isolation of the endoplasmic reticular fraction aids in immunoblotting analysis of CYP by enriching CYP content and allows the isolation of active enzymes (Guengerich, 1990; Prough et al., 1977). CYP enzymes have been characterized historically by their content in the microsomal fraction and are sometimes difficult to detect if only cell lysates are analyzed. Recently, other CYP enzymes have been studied in human lung, including CYP2E1, CYP2B7, CYP4B1, CYP2D6, CYP3A4, and CYP3A5 (Czerwinski et al., 1994; Shimada et al., 1996b; Willey et al., 1997). CYP2D6 mRNA and protein has been found at nonexistent to very low basal levels in human lung by reverse transcription-polymerase chain reaction (RT-PCR) and immunoblotting techniques (Guidice et al., 1977; Kivisto et al., 1997). CYP2B7 and CYP4B1 have been described by mRNA expression (Czerwinski et al., 1994; Willey et al., 1997). CYP3A enzymes have been found by immunohistochemistry in normal human lung and lung tumor tissues, although RT-PCR analysis detected only CYP3A5 (Kivisto et al., 1995). Another report also concluded that CYP3A5 was the major CYP3A isoform in human lung because CYP3A4 was only detected in one of the eight lung samples examined (Anttila et al., 1997). However, activity of these enzymes toward major tobacco carcinogens such as B[a]P is low in comparison to enzymes of the CYP1 family (Bauer et al., 1995; Gautier et al., 1996; Shou et al., 1994). CYP1A1 has been studied in human lung by its activity, mRNA expression, and immunohistochemistry (Karki et al., 1987; McLemore et al., 1990; Saarikoski et al., 1998; Sipal et al., 1979; Stoner et al., 1978; Wheeler et al., 1990). However, there has yet to be clear quantitative data describing both CYP1A1 and CYP1B1 proteins in the same human lung samples.

CYP1A1 showed considerable interindividual variation, ranging from 4.3 pmol/mg microsomal protein to 289 pmol/mg microsomal protein. One-way ANOVA showed a statistically significant difference between smokers and non-smokers, and ex-smokers and non-smokers. This difference most likely reflects the induction of CYP1A1 by PAHs in tobacco smoke (Nebert et al., 1991; Okey, 1990). CYP1B1 showed much less expression in human lung, ranging from 0.3 to 12.7 pmol/mg microsomal protein. Although the comparison between smokers and non-smokers was not statistically significant, smokers and ex-smokers are higher than non-smokers, following the same trend as seen with CYP1A1. In contrast to a recent study by Wei et al. (2001), which used RT-PCR analysis of total lung RNA, we did not detect the expression the CYP1A2.

Of interest, CYP protein expression measured in ex-smokers showed levels that are similar to those seen in smokers. Possibly, long-term smoking established a phenotypic alteration (Niewoehner et al., 1974) leading to continued induction of CYP or deposits of tobacco–smoke tar, and debris provides a continual source of Ah receptor-activating ligand. Although these hypotheses need to be more fully explored, this novel observation of sustained elevations of CYP1A1 and CYP1B1 in lung tissue of ex-smokers could explain why the risk of cancer, although decreased in ex-smokers, does not return to baseline. This may be especially important in individuals exposed to occupational sources of carcinogens similar to those found in tobacco smoke. However, it must be noted that smoking prevalence can be significantly underestimated by self-report when compared to that defined by measures of blood

cotinine levels. This source of bias is especially high in ex-smokers (Lewis et al., 2003), and thus these results should be viewed cautiously.

Our observation of constitutive and inducible expression of CYP1B1 in lung tissue samples is in contrast with an earlier study by Hakkola et al. (1997) where CYP1B1 expression was not detected in any of the adult lung tissue samples tested by RT-PCR. Our study is in agreement with another study (Willey et al., 1997), again by RT-PCR, showing the elevation of CYP1B1 expression in bronchial epithelial cells of smokers versus non-smokers. However, both this study (Willey et al., 1997) and a later study (Pipari et al., 2000) were able only to detect CYP1B1 RNA in a small percentage of the samples analyzed. The later study (Pipari et al., 2000) and another (Spivak et al., 2001) also reported immunoblot detection of CYP1B1 protein in a fraction of the cell (Pipari et al., 2000) or tissue (Spivak et al., 2001) samples that were analyzed, but the sensitivity of the assays used in these studies did not permit the protein levels to be quantified. Perhaps one of the most important findings of the current study is the consistent expression of CYP1B1 in normal tissue from organ donors. The demonstration of CYP1B1 protein expression reported here provides independent confirmation of the immunohistochemical study by Muskhelishvili et al. (2001), which used a different CYP1B1 antibody and showed the expression of CYP1B1 protein in several normal human tissue samples. Furthermore, the current study expands the knowledge of normal expression of CYP1B1 to the lung and measures, for the first time, specific protein content of CYP1B1 by immunoblot analysis. The observed expression of CYP1B1 in normal human tissue is in stark contrast to other studies based on the analysis of tumor biopsy tissue that reported the tumor-specific expression of CYP1B1 (Murray et al., 1997). It is now clear that CYP1B1 is expressed at low levels in a variety of tissues and that its level of expression may be increased in tumor tissue, or in normal tissue in response to exposure to PAHs.

Correlation analysis shows a significant positive association between CYP1A1 and CYP1B1 expression in smokers. This agrees with mechanistic data showing the regulation of these genes by the Ah receptor (Landers and Bunce, 1991; Sutter et al., 1991, 1994; Tang et al., 1996; Whitlock, 1999; Whitlock et al., 1996). This correlation may reflect Ah receptor genetics and polymorphisms (Hankinson, 1995; Okey, 1990; Poland and Knutson, 1982; Swanson and Bradfield, 1993). Ah receptor polymorphisms can account for differences in inducibility of CYP1 enzymes. Approximately 10% of the population exhibits high induction of aryl hydrocarbon hydroxylase activity (Nebert et al., 1991), which may increase risk of lung cancer (Kellermann et al., 1973a, 1973b, 1980; Kouri et al., 1982; Nebert, 1991; Nebert et al., 1999). Those individuals with high CYP expression may possess the greatest risk for lung cancer because of smoking. The simultaneous analysis of both CYP1A1 and CYP1B1 may be utilized in the identification of those individuals that are high responders to exposures to dioxin-like molecules.

In this correlation analysis, CYP1A1 and CYP1B1 failed to show significant association among the ex-smokers (Fig. 3). This suggests that other factors may affect the independent induction and expression of CYP1A1 and CYP1B1 after the exposure to Ah receptor agonists ceases.

CYP1B1 is expressed at low levels compared to CYP1A1 in human lung but may still play major roles in the bioactivation of sterically hindered PAHs. In animal models, CYP1B1 plays a major role in the carcinogenicity of DMBA. CYP1B1-null mice, which maintain expression of CYP1A, reportedly show resistance to DMBA-induced lymphomas (Buters et al., 1999). DB[*a,l*]P is another sterically hindered PAH found in the environment and tobacco smoke, and has been shown to possess high mutagenic activity in mouse skin and high carcinogenicity in rat mammary gland models (Cavalieri et al., 1991). Recombinantly expressed human CYP1B1 has been shown to metabolize DB[*a,l*]P to detectable DB[*a,l*]P-diol-epoxide-DNA adducts levels 4- to 10-fold greater than human CYP1A1 (Luch et al., 1998). Using the SOS response of *Salmonella typhimurium* tester strains as an endpoint of DNA damage, CYP1B1 showed extremely high activity for conversion of DB[*a,l*]P-11,12-diol, B[*a*]P-7,8-diol, DMBA-3,4-diol, and 5-methylchrysene to mutagenic metabolites (Shimada et al., 1996a). Though CYP1B1 levels are much lower than CYP1A1 in human lung, CYP1B1 may play an important role in the bioactivation of sterically hindered PAHs and proximate carcinogens.

Sample size calculations for future studies of CYP1B1 expression indicate that approximately double the sample sizes used in this report (smoker $n = 36$, non-smoker $n = 14$, and ex-smoker $n = 14$) are necessary to achieve statistically significant ANOVA and pairwise comparisons.

In this report, we could not vigorously pursue CYP activity in these human lung microsomal fractions because of low activity and limited amount of microsomal protein. Although there are reports of detectable aryl hydrocarbon hydroxylase, ethoxycoumarin-*O*-deethylase, and B[*a*]P and B[*a*]P-7,8-dihydrodiol metabolism activities of human lung microsomes, these studies have also reported very low levels of cytochrome P450-associated activity (McManus et al., 1980; Prough et al., 1977, 1979; Rojas et al., 1992; Shimada et al., 1992, 1996b; Sipal et al., 1979; Wheeler and Guenthner, 1991; Wheeler et al., 1990). In many cases, CYP has not been detectable and has been estimated to be 10% of the CYP content of human liver (Guengerich, 1990; Jakobsson et al., 1982; Lorenz et al., 1979; McManus et al., 1980; Shimada et al., 1994; Watanabe and Abe, 1981). An early study also reported that human lung microsomes appeared to contain an inhibitory element because these microsomes inhibited the ethoxycoumarin-*O*-deethylase activity of rat liver microsomes in a concentration-dependent manner (Lorenz et al., 1979). The detection of human lung CYP protein and activity is considerably more difficult than in

animal tissues because of the much lower levels of CYP in human lung. In addition, factors such as post-mortem handling of human tissues and delay-time before freezing may greatly affect the stability of CYP in human tissues (Jakobsson et al., 1982).

The expression of CYP in specific lung cell types can further our understanding of the progenitor cells involved in the initiation of the multistage chemical carcinogenesis process (Shields and Harris, 1991). The most common cell types of lung carcinoma are squamous cell carcinoma, small cell carcinoma, adenocarcinoma, large cell carcinoma, and adenosquamous carcinoma (Hasleton and Spencer, 1996). The high incidence of squamous cell carcinoma among male smokers initially led researchers to postulate that tobacco smoking was causally related to the development of squamous cell carcinoma. Among young patients, particularly non-smokers and females, adenocarcinoma is predominant compared to the other carcinomas (Pass, 1996). This led to hypotheses that adenocarcinoma was not a tobacco-related cancer. However, current epidemiological evidence has shown increases in adenocarcinoma, as well as in squamous cell carcinoma, in women, corresponding to increases in smoking rates (Samet, 1994). Ultrastructural evidence has shown that adenocarcinomas contain characteristics of Clara cells and alveolar type II pneumocytes, cell types known to contain the CYP monooxygenase system in human lung and animal lung models (Anttila et al., 1991, 1997; Devereux, 1984; Devereux and Fouts, 1981; Devereux et al., 1989; Hasleton and Spencer, 1996). Squamous cell carcinoma is thought to be derived from bronchial epithelium although small cell carcinoma is believed to be of neuroendocrine origin (Shields and Harris, 1991). On the other hand, large cell carcinomas are a heterogeneous group of tumors encompassing squamous, glandular, and neuroendocrine characteristics (Hasleton and Spencer, 1996). From animal model studies, it has been hypothesized that because Clara cells and alveolar type II cells are the primary cell types expressing CYP, tobacco smoking must be related to adenocarcinoma. However, human lung carcinomas often show characteristics of more than one type of differentiation, and have also been shown to change differentiation characteristics during lung tumor progression (Pass, 1996). Therefore, a unifying scheme of lung histogenesis based on similarities in cell types between tumor and normal tissues is largely conjectural until more research is accomplished.

The data presented here clearly show CYP1A1 to be one of the major CYP enzymes in human lung. However, lack of strong quantitative data on other isoforms, such as CYP3A, CYP2B7, and CYP4B1, prevents us from determining if CYP1A1 is the major isoform expressed. Knowledge about the metabolic capacity of CYP1A1 for PAHs, however, allows us to conclude that it is primarily responsible for metabolism and activation in human lung of many procarcinogens and xenobiotics present in the environment and tobacco smoke. Levels of CYP expression show high interindividual variation. Immunohistochemical data presented here confirm earlier reports of CYP1A1 expression in Clara cells, alveolar type I and type II cells, ciliated columnar epithelial cells, and macrophages. In addition, we have shown CYP1B1 expression to be in the same cell types expressing CYP1A1. Though more research into the histogenesis of lung carcinomas needs to be accomplished, it is believed that Clara cells and alveolar type II cells are potential precursors to adenocarcinoma, a lung cancer linked to tobacco smoking. In addition, bronchial epithelium-encompassing cell types such as ciliated columnar cells are thought to be potential precursors to squamous cell carcinoma. We show here that each of these cell types expresses CYP1A1 and CYP1B1, enzymes that are likely to play a significant role in the activation of procarcinogens found in cigarette smoke. CYP1A1 induction observed in smokers and ex-smokers, in addition to greater exposure to carcinogens, may account for cancer susceptibility among those individuals who are high responders.

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